1.a. **Full Title**: A Meta-Analysis of Expanded Genome-wide Association Studies on high-sensitivity Troponin T and I
   
   **b. Abbreviated Title (Length 26 characters)**: Troponins Meta-GWAS

2. **Writing Group**:
   Writing group members: Yunju Yang, Michael Brown, Ron C. Hoogeveen, Christie M. Ballantyne, Eric Boerwinkle, Goo Jun and Bing Yu

Others are welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __YY___ [please confirm with your initials electronically or in writing]

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3. **Timeline**:
   - Completion of GWAS results: 15th Aug, 2019
   - Completion of Meta-analysis: 15th Oct, 2019
   - Completion of Mendelian Randomization analysis: 15th Dec, 2019
   - Completion of writing a Manuscript: 15th Mar, 2020

4. **Rationale**:
The troponin complex was discovered over forty years ago and has been identified as a cardiac biomarker in relation to coronary heart disease (CHD) (Beatty et al., 2013; Saunders et al., 2011; Zethelius, Johnston, & Venge, 2006). Since they exhibit high sensitivity and near perfect specificity, troponin biomarkers have been declared as the preferred markers for diagnosing cardiac diseases by the American College of Cardiology and the European Society of Cardiology (Amsterdam et al., 2014). Highly sensitive troponin measurements have been reported to be associated with adverse cardiovascular events in diabetes (Lepojarvi et al., 2016) and heart failure (HF) (Latini et al., 2007). ARIC investigators have reported that individuals with a high troponin T level were at higher risk of developing CHD (Saunders et al., 2011). Both troponin T and I have been shown to have added value to a CHD prediction model (Blankenberg et al., 2016; Hochholzer et al., 2014), genetic variations contributing to troponins may improve the understanding of CHD risk. A genome-wide association study (GWAS), including ARIC, has identified one genome-wide significant locus at 8q13.3 to be associated with troponin T (Yu et al., 2013), however, the genetic effect of troponin I has not been examined and the sample size is relatively small. Here we propose to analyze imputed genotypes with sequence-based reference panels to test genetic associations in a larger set of samples to promote additional genetic discoveries for troponin markers. Additionally, we propose to estimate the potential troponin causal effect on CHD using Mendelian randomization (MR) approaches.

5. Main Hypothesis/Study Questions:
   1. To identify common genetic variants associated with troponin T and troponin I;
   2. To test the potential troponin causal effect on CHD via MR approaches

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

This is a meta-analysis between multiple studies, including ARIC. The methods below describe the detailed analysis in ARIC and general approaches of meta- and MR analysis.

ARIC collected plasma-based troponin measurements at the visit 4 from 10,350 participants using the Elecsys Troponin T high sensitivity assay [Roche diagnostics, Indianapolis, IN] and the chemiluminescent troponin I immunoassay [Abbott, Architect Stat Troponin-I]. The detection limit of assay is 3 ng/L and measurement values under detection limit were converted to a half of the limit. All troponin levels were treated in ng/L unit. Troponin levels are treated as 1) a continuous variable with inverse-normalization, and 2) a binary variable with a race-gender specific 99% percentile threshold.

Exclusion Criteria
   1) Subject without informed consent on DNA use
   2) Subjects without demographic (i.e. race, center and sex) and troponin information
   3) Prevalent CHD or HF cases at the time of troponin measurements

Statistical analyses

QC procedures
Genotype data on SNP array was imputed on the 1000 Genome Reference panel (phase 1 version 3). Prior to GWAS, SNPs with imputation quality less than 0.3 and/or population-specific MAF less than 0.5% were excluded. Only SNPs on autosomes will be included in this project.

**Cohort-level GWAS**

Linear and logistic models for association with individual SNPs will be applied under additive genetic model adjusting for age, sex, study site and 10 race-specific principal components. Analysis will be performed separately according to ethnicities.

**Meta-analysis**

Currently, the meta-analysis is planned to combine the summary statistics from seven individual cohorts (AGES, ARIC, CHS, MESA, PROSPER, SHIP and SHIP_Trend). Additional studies may be included as the project moves along. Fixed-effect inverse variance meta-analyses will be applied. Bonferroni correction method will be used to define the significance as p-value<5xe-8.

**Two-sample Mendelian randomization**

We will use R-package 2SMR to perform a two-sample MR analysis using statistics from CHD GWAS (i.e. UK Biobank and CARDIoGRAMplusC4D (Nelson et al., 2017)) and troponin associations identified in this project. Bonferroni correction method will be applied to MR p-values with the number of troponin variants tested.

7.a. Will the data be used for non-CVD analysis in this manuscript? ___ Yes  __X__ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? ___ Yes  ___ No
   (This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  _X_ Yes  ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  _X_ Yes  ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications list under the Study Members Area of the website at: [http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html](http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html)

   ___X___ Yes  _______ No
10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

MS# 1668, “Genome wide association study (GWAS) for Novel highly sensitive cardiac Troponin-T (hs-cTnT) levels in the ARIC Study”

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes    ___ No

11.b. If yes, is the proposal  
   ___ A. primarily the result of an ancillary study (list number* __________)
   ___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ ____________)

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PubMed Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.
References